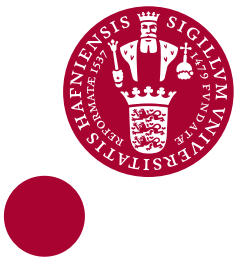

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Extent of natural attenuation of chlorinated ethenes at a contaminated site in Denmark

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Abstract

Chlorinated solvents contaminated soils and aquifers are a widespread problem in industrialized countries and many require clean-up due to the risk of contaminant flow into groundwater systems. Clean-up is costly and often invasive, thus there is high interest in stimulating natural attenuation processes. For this, first an assessment of the type and extent of natural attenuation present at the site is required. Here, we present chemical, isotopic and microbial analyses of waters collected at a chlorinated ethene contaminated site in Denmark to give insights into natural attenuation processes. The data gives indication of complete reductive dechlorination by microbial communities but their extent varies greatly across short distances and between the different geological layers. The data further indicates that overall, chlorinated ethene degradation through natural attenuation is small at this site but near surface degradation due to aerobic co-metabolism or abiotic geochemical reduction could potentially play a role.

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Keywords: Chlorinated solvents; remediation; compound stable isotope analysis; ^{13}C

1. Introduction

The extensive use of chlorinated solvents, particularly perchloro- and trichloro-ethylene (PCE, TCE), as cleaning and degreasing agents, together with their long unrecognized harmful potential, has led to their widespread release into soils and aquifers. Even at low concentrations (< 5 µg/l) they are considered harmful because of their persistence and carcinogenic potential, rendering them lasting threats for groundwater security and human and environmental health. Their cleanup is particularly challenging and also costly, due to their hydrophobicity, leading

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to long lasting dissolution processes as well as ad- and desorption to/from clay and organic carbon matrices. Over the past decades considerable effort has been undertaken to remediate these contaminated sites [1], mainly by excavation followed by treatment of the soil material *ex situ* (if contamination is close to the surface), or by pump and treat (for deep lying or expansive contamination).

In certain cases, natural attenuation processes, specifically anaerobic microbial reductive dechlorination, can substantially aid in the cleanup of PCE and TCE spills, or in some cases even fully remediate them. It is well established that members of microbial phyla, such as *Firmicutes*, *δ*- and *ε*-*Proteobacteria*, can sequentially reduce PCE to TCE and dichloroethylene (DCE, mostly cis - cDCE) via a respiratory pathway [2], [3]. Additionally, the discovery that species from the phylum *Chloroflexi*, particularly the genus *Dehalococcoides* (DHC) and potentially *Dehalogenimonas* (DHG), are capable to dechlorinate PCE and TCE past DCE via vinylchloride (VC) to ethylene (ETH), has sparked hopes for natural biological processes to achieve complete cleanup [4]–[8]. However, to assess the potential for biological attenuation, it is key to know the microbial community structure and determine if species of *Dehalococcoides* and *Dehalogenimonas* are both present and active. Note that aerobic co-metabolism of lower chlorinated ethenes (e.g., DCE and VC; does not work for PCE) is generally considered of low importance, because bacteria do not gain energy from this process [9], [10].

Another means to assess the type and extent of natural attenuation is through the use of compound specific isotope analysis (CSIA) [11]. For example in reductive dechlorination (RD), a reaction specific kinetic isotope effect leads to an enrichment of the heavier isotopes (^{13}C and ^{37}Cl) in the parent compound and a "lighter" fraction in degradation products. Combining the method for both C and Cl leads to two dimensional (2D) isotope plots that can provide enhanced discrimination of ongoing processes [12], [13]. Furthermore, as biological RD proceeds sequentially, the total amount of ^{13}C is conserved until complete dechlorination occurs. Therefore a mass balance over this isotope can provide information of the overall mass loss, if the virgin ^{13}C signature is known. For a review of CSIA of chlorinated ethenes please refer to a recent report [14].

In this study, chemical, microbiological and isotopic techniques were applied to evaluate the natural attenuation potential at a chlorinated ethene contaminated site in Denmark. The study illustrates how attenuation processes vary greatly across small scales and how a wealth of information can be obtained from this multidisciplinary approach.

2. Methods

2.1. Site description

The study site is a former dry-cleaning facility (two buildings, Fig. 1) in Skovlunde, Denmark. From 1960-1987, approximately 2 tons of PCE were gradually released into the underlying ground, which consists of a saturated 6-8 m clay layer overlying the primary sandy aquifer, with the water table situated at around 13 m depth. Contamination has been investigated since 1997 revealing high PCE and TCE contamination in both the clay layer and the underlying aquifer [15]. BTEX (Benzene, Toluene, Ethylbenzene, Xylene) and hydrocarbons are also present.

2.2. Groundwater sampling and analysis

Groundwater samples were collected from wells B11, B111 and B12 located in the clay layer plus B56 and P01 in the aquifer during two campaigns in February and April 2017. An electric dive pump was used and prior to sample collection, at least three well casing volumes were pumped to obtain representative samples. The only exception was B111, where the well was pumped and sampled until exhausted. Samples for chemical analysis were filled in glass vials with excess overflow and were capped with septa lined screw caps without headspace. Samples

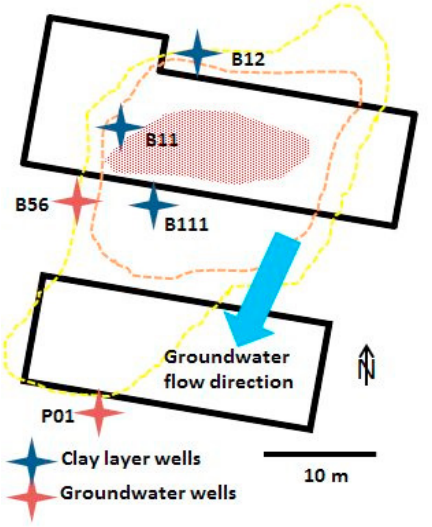

Fig. 1. Schematic depiction of the contaminated site, relevant wells. Qualitative distribution of contamination from previously existing data is shown as source area (red area) and lower concentration plume extent (orange and yellow dashed lines) as over different depths.

were stored on ice and sent to Eurofins Denmark A/S for analysis. Samples for isotope analysis were taken in a similar manner but immediately after collection, two sodium hydroxide pellets were added to stop any microbial degradation. Glass bottles were capped with screw caps containing Teflon coated silicon liners and stored at 4°C. Samples were sent to Isodetect GmbH for ^{13}C and ^{37}Cl isotope analysis by isotope ratio mass spectrometry (IRMS). Results are expressed in the δ-notation as ‰ relative to the international reference standards VPDB (Vienna Pee Dee Belemnite) and SMOC (standard mean ocean chloride) for ^{13}C and ^{37}Cl, respectively. Samples for microbial analysis were taken in 1L polypropylene bottles and immediately shipped on ice to Microbial Insights BV (Belgium) for quant-array Chlor assay. This assay uses a qPCR approach to enumerate the number of several known degraders of chlorinated ethenes (anaerobic reductive, aerobic co-metabolic) along with methanogens, sulfate reducers and total eubacteria.

3. Results and Discussion

3.1. Chemical lines of evidence

Chemical analyses showed that the majority of contamination was retained in the clay layer (B11, B111 and B12; Table 1) and this formation can be considered the main source for gradual PCE dissolution (i.e., release) into the underlying layers. Among these three clay sampling spots, variations exist in terms of total contaminant mass and molar fraction of constituents (Table 1; Fig. 2). B11 and B12 are dominated by PCE, as expected for an undisturbed source zone, while B111 exhibits a high abundance of degradation products, i.e., cDCE, thus indicates a hot spot for reductive dechlorination. This is also matched by the observed redox conditions: aerobic conditions in B11 and B12 (presence of nitrate and nitrite; unfavorable of reductive dechlorination) and reduced conditions in well B111 (presence of Fe(II) and methane).

Among several wells in the aquifer (i.e., below groundwater table) contaminant concentrations were generally low (usually <5 µg/L, probably due to the on-going pump and treat, P&T), except for the wells B56 and P01 that are explicitly considered for this study (Table 1). B56 is characterized by a high abundance of degradation products, fairly high concentrations of hydrocarbons and BTEX compounds, and iron reducing conditions (Fig. 1); all together indicative of the presence of biological reductive dechlorination. The groundwater well P01, feeding the P&T facility, shows similar geochemical conditions but concentrations are substantially lower compared to B56. This is explained by the catchment of the P&T exceeding the contaminated area (i.e., diluting contaminated groundwater with uncontaminated water). It reflects a grand average of the P&T catchment area (groundwater system) in terms of relative abundance of chlorinated ethenes and extent of degradation.

Table 1. Concentrations of contaminants PCE, TCE, cDCE, VC and sum of co-contaminants BTEX and hydrocarbons (C_6-C_{35}) in µg/l, as well as redox parameters (mg/l) in selected wells. n.d. = not detected.

	Clay layer			Groundwater	
	B11	B12	B111	B56	P01
PCE	62000	35000	640	71	16
TCE	2200	1800	710	69	5.8
cDCE	1500	1400	8800	100	6.3
VC	65	74	590	n.d.	n.d.
Σ BTEX	3.7	1.2	1.9	<0.1	n.d.
Σ C_6-C_{35}	14000	7700	250	45	7
NO3	67	8.9	n.d.	n.d.	n.d.
NO2	0.46	0.099	n.d.	n.d.	n.d.
NH_3	0.32	0.04	0.02	0.11	0.13
Fe(III)	0.12	0.96	37	0.26	0.15
Fe(II)	n.d.	0.012	0.022	12	3.9
Sulfide	n.d.	n.d.	n.d.	n.d.	n.d.
CH_4	0.008	n.d.	0.039	n.d.	0.032

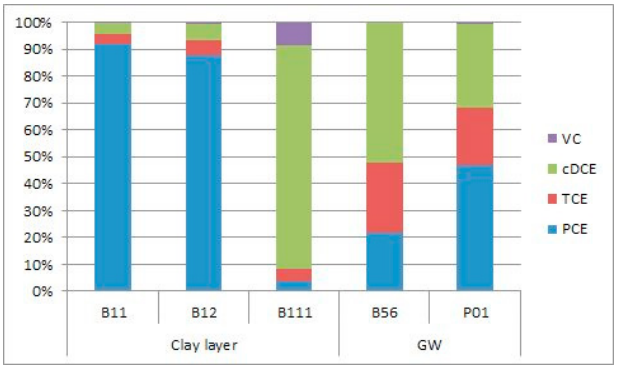

Fig. 2. Molar fraction of chlorinated ethenes in selected wells of the clay layer and groundwater (GW).

3.2. Microbiological lines of evidence

Microbial gene and enzyme analyses were performed for sampling wells indicative of biological RD (i.e., B111, B56). Overall, these showed the presence of *Dehalococcoides* and also *Dehalogenimonas* species and the necessary genes for complete degradation to ethenes (i.e., BVC VCR, Table 2). Thus, there is potential for complete dechlorination to occur both in the clay layer and in the aquifer/groundwater. The cell numbers of degraders carrying out incomplete dehalogenation, i.e., PCE to cDCE only (*e.g.* DHBt, DSB, DSM; Table 2) was however, substantially higher, which likely indicates that this is the dominant process. Generally, microbial activity seems higher in the aquifer/groundwater, albeit the cell number itself is not necessarily correlated to activity. Aside from reductive dehalogenators, bacteria that co-metabolize lower chlorinated ethenes (TCE, DCE, VC) with BTEX compounds and methane were also observed. Such processes are not expected from chemical analyses but could also be taking place at the site.

Table 2. Number of cells and key enzymes detected in wells B111 and B56 by qPCR microarray. bdl = below detection limit, a = below practical quantification limit but above lower quantification limit, GW = groundwater.

	Clay	GW
Bacteria/enzymes	B111	B56
Dehalococcoides (DHC)	16	$3.1\ 10^2$
tceA Reductase (TCE)	0.7 [a]	bdl
BAV1 Vinyl Chloride Reductase (BVC)	0.9[a]	2.2[a]
Vinyl Chloride Reductase (VCR)	1.7	22
Dehalobacter spp. (DHBt)	3.3×10^2	6.1×10^3
Dehalogenimonas spp. (DHG)	2.7×10^2	1.7×10^5
Desulfitobacterium spp. (DSB)	66	1.1×10^4
Desulfuromonas spp. (DSM)	5.2	9.3×10^2

3.3. Isotopic lines of evidence

To further constrain the nature and extent of natural attenuation, $\delta^{13}C$ and $\delta^{37}Cl$ isotopic analyses of waters from the two degradation hot spots (B111, B56) and from the undisturbed source (B11) were performed. Values are depicted as dual isotope plot in Fig. 3, together with known source ranges for PCE and TCE [16]–[19].

The most negative $\delta^{13}C$ value of PCE (i.e., the least dechlorinated compound) was found in well B11. This value falls within analytical error of PCE source material (Fig. 3), thus matches our interpretation of B11 resembling the source zone.

Overall, a well by well consideration shows that TCE is more enriched in $\delta^{13}C$ and $\delta^{37}Cl$ than PCE, and is also far from the range of the considered source material. This suggests that in all three considered wells TCE is actively degraded, even in the B11 source zone well showing little amounts of cDCE. The stronger fractionation in ^{37}Cl than in ^{13}C is expected for isotope fractionation by biological RD, according to reported fractionation factors [14].

Compared to TCE, the next degradation product cDCE marks much less enriched compounds in both B111 and B56. This implies that cDCE is accumulated as a product, rather than being depleted. Moreover, it suggests that reductive dechlorination is stalled at this stage, as would be expected from the fairly high abundance of incomplete microbial degraders (Table 2). Only the ^{37}Cl fraction in well B111 is more enriched than in the parent compound (TCE), showing that some degradation of cDCE is occurring (VC observed, Fig. 2). Surprisingly however in source zone well B11, cDCE displays a much more positive signature in both elements than the parent compound TCE. This suggests strong fractionation due to degradation. Because this well is oxygenated, degradation is interpreted to occur via aerobic oxidation without VC as intermediate and the observation of little cDCE in this well might be due to rapid removal. This is also supported by the presence of microorganisms known for aerobic co-metabolism at the site (wells B111 and B56) as well as BTEX compounds that are common main metabolites of aerobic co-metabolism. In the past however, similar geochemical signatures have been interpreted to reflect abiotic (geo-)chemical reduction [20]–[23]. In principle, a process discrimination via dual isotope slopes could be possible (several fractionation factors reported for ^{13}C and few for ^{37}Cl), as long as masking effects (e.g. diffusion limitation) are negligible [11]. However, due to the limited number of data points from the field, further discrimination is not possible. Obtaining isotopic data of cDCE in well B12 and other aerobic parts could help to constrain the process that is responsible for cDCE removal.

For PCE the strongest isotopic enrichments, *i.e.* extent of degradation, is seen in the groundwater from well B56. The pump and treat well, P01, contains the site hydraulically and should represent an average, overall process. The lower isotopic enrichments in P01 compared to B56 indicate that the overall progress of biological RD at the site is less pronounced and that the investigated water at B56 resembles a "hot-spot".

To estimate the total loss of chlorinated ethenes a mass balance over the heavier carbon isotope can be calculated. To do this, for each detected/measured chlorinated ethene, the $\delta^{13}C$ value (Fig. 3) is multiplied with its respective molar ratio (Fig. 2) and these values are then added. If the sum remains constant, there is no loss. If not, ^{13}C might have left the system as ethene (complete biological RD), acetylene (abiotic RD) or CO_2 (aerobic cometabolism). The marginal differences in the $\delta^{13}C$ mass balance among the three wells (Table 3) imply that overall degradation due to RD is limited compared to removal from the appointed source zone (B11). Further sampling and analyses are needed to confirm the observed trends, particularly in the aerobic wells, and to obtain a more representative source material (i.e., less degraded PCE).

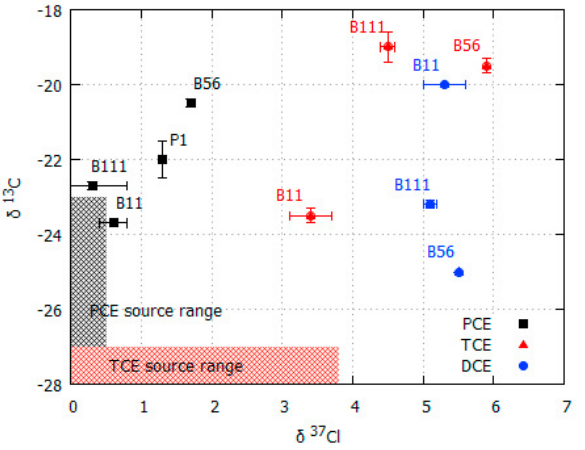

Fig. 3. 2D-isotope plot of $\delta^{13}C$ and $\delta^{37}Cl$ in waters collected from wells in the clay layer (B11, B111) and groundwater (B56). Error bars represent analytical standard deviations. The black and red marked areas designate the range of virgin source signatures commonly considered for PCE and TCE, respectively.

Table 3. $\delta^{13}C$ mass balances (MB) for key wells expressed in ‰ to the reference standard VPDB. GW = groundwater.

	Clay layer		GW
	B11	B111	B56
$\delta^{13}C$-MB	-23.5	-23.0	-22.4

4. Conclusion

Overall this study highlights the complexity of natural attenuation processes at chlorinated ethenes contaminated sites and it also illustrates the importance of a multidisciplinary approach to gain a clearer understanding of ongoing processes. While both partial and complete biological dechlorination seems possible and even highly active at certain sites (hot spots), they seem less active at other, showing a strongly heterogeneous pattern of processes occurring at short scales. Multiple lines of evidence were needed to confirm intrinsic degradation potentials. The isotopic line of evidence was necessary to reveal the overall limited extent of degradation via biological RD but also suggests the possible occurrence of aerobic degradation, which was not expected. Further analyses are needed to help constrain processes in the clay layer, to identify other potential hot spots and eventually obtain less degraded portions of source contaminant to allow for an estimation of loss due to aerobic pathways.

Acknowledgements

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